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Effect of poly and mono-unsaturated fatty acids on stability and structure of recombinant S100A8/A9.

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Abstract

Recombinant pET 15b vectors containing the coding sequences S100A8 and S100A9 are expressed in Escherichia coli BL21 (DE3) and purified using Ni-NTA affinity chromatography. The structural changes of S100A8/A9 complex are analyzed upon interaction with poly/mono-unsaturated fatty acids (UFAs). The thermodynamic values, Gibbs free energy and the protein melting point, are obtained through thermal denaturation of protein both with and without UFAs by thermal scanning of protein emission using the fluorescence spectroscopy technique. The far-ultraviolet circular dichroism spectra show that all studied unsaturated fatty acids, including arachidonic, linoleic, alpha-linolenic and oleic acids, induce changes in the secondary structure of S100A8/A9 by reducing the α-helix and β-sheet structures. The tertiary structure of S100A8/A9 has fluctuations in the fluorescence emission spectra after the incubation of protein with UFAs. The blue-shift of emission maximum wavelength and the increase in fluorescence intensity of anilino naphthalene-8-sulfonic acid confirm that the partial unfolding is caused by the conformational changes in the tertiary structure in the presence of UFAs. The structural changes in S100A8/A9 and its lower stability in the presence of UFAs may be necessary for S100A8/A9 to play a biological role in the inflammatory milieu.

KEYWORDS: Circular dichroism; S100A8/A9; Stability; Thermal denaturation; Unsaturated fatty acid

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